SHOCK, Vol. 37, No. 6, pp. 605 610, 2012

DAILY PROFILES OF FIBRINOGEN METABOLISM FOR 5 DAYS FOLLOWING HEMORRHAGE AND LACTATED RINGER'S RESUSCITATION IN PIGS

Wenjun Z. Martini, Kevin K. Chung, Michael A. Dubick, and Lorne H. Blackbourne

US Army Institute of Surgical Research, Ft Sam Houston, Texas

Received 1 Feb 2012; first review completed 22 Feb 2012; accepted in final form 24 Feb 2012

ABSTRACT This study's objective was to investigate the daily dynamics of fibrinogen metabolism and coagulation function for 5 days after hemorrhagic shock in pigs. Sixteen pigs were randomized into the control (C) and the hemorrhage (H) groups. On day 1, hemorrhage was induced in H by bleeding 35% of the estimated total blood volume, followed by resuscitation with lactated Ringer's solution at three times the bled volume. Then, a primed constant infusion of stable isotopes was performed in both groups daily for 5 days to measure changes in fibrinogen metabolism, together with changes in hemodynamics and coagulation function. Hemorrhage caused a decrease in mean arterial pressure and an increase in heart rate. Fluid resuscitation corrected these changes. Compared with baseline day 1, fibrinogen levels in H were decreased to 76% ± 6% by hemorrhage and resuscitation on day 1, increased to 217% ± 16% on day 2, and remained elevated afterward; clot strength in H was decreased by hemorrhage on day 1 and returned to baseline values on day 2 and afterward. Compared with day 1 control value (1.3 ± 0.1 mg/kg per hour), fibrinogen synthesis in H was increased to 3.6 ± 0.1, 5.1 ± 0.5, 2.6 ± 0.4, 2.7 ± 0.5, and 2.3 ± 0.3 mg/kg per hour on days 1 through 5 (all $P < 0.05$); fibrinogen breakdown in H was elevated on days 1 and 2 but returned to control values afterward. Hemorrhage caused acute decreases in fibrinogen concentration and clot strength, followed by an increase in fibrinogen concentration and recovery of clot strength. The increase in fibrinogen appeared primarily due to a sustained increase in fibrinogen synthesis.

KEYWORDS Hemorrhagic shock, fibrinogen synthesis, fibrinogen breakdown, coagulation, stable isotopes, gas chromatograph mass spectrometry

INTRODUCTION

Hemorrhage is the leading potentially preventable cause of death on the battlefield and a major cause of death in civilian trauma (1, 2). Following blood loss, all components involved in the coagulation process are reduced and further diluted by resuscitation with crystalloid or colloid fluids. To restore coagulation function, different blood products, such as platelet concentrates, cryoprecipitate, or fresh frozen plasma, have been used in patients with bleeding complications (3–5). However, limited information is available to justify the priority of supplementing hemostatic components.

Among all coagulation components, fibrinogen is the first to drop to a critical level after trauma and hemorrhage (6), compromising clot strength and coagulation function. On the other hand, elevated fibrinogen levels were commonly observed in patients days after traumatic injury or surgery (7–9), attributed to the acute-phase response. However, the underlying mechanisms related to the dynamic shift of fibrinogen concentration remain unclear. The increase in fibrinogen concentration might result from accelerated hepatic synthesis, decreased breakdown, or both, or fluctuated plasma volume. The last point is especially valid after hemorrhagic shock, because fluid resuscitation is often used as routine clinical care to restore vascular function and improve tissue perfusion. Different resuscitation fluids, including crystalloids, colloid fluids, and blood products, may change plasma volumes differently in response to different osmolar or oncotic pressures. Thus, to reveal underlying mechanisms contributing to changes in fibrinogen concentration, it is necessary to investigate changes in fibrinogen synthesis, degradation, and plasma volume simultaneously and independently.

This study was designed to test the hypothesis that the increase in fibrinogen concentration after hemorrhage and resuscitation results from the increase in fibrinogen synthesis. Daily changes of fibrinogen metabolism were quantified for 5 days after hemorrhagic shock and resuscitation in a swine model. Upon the induction of hemorrhage and resuscitation with lactated Ringer's (LR) solution on day 1, endogenous fibrinogen synthesis rates and breakdown rates were quantified daily for 5 days, using a 6-h stable isotope infusion with subsequent gas chromatography and mass spectrometry analysis as previously described (10–12). Changes in plasma volumes were measured daily for 5 days during the isotope infusion. Corresponding changes in coagulation function were assessed daily to correlate changes of fibrinogen availability after hemorrhagic shock and LR resuscitation.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research and has been conducted in compliance with the Animal Welfare Act and the implementing Animal Welfare Regulations and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals. A total of 16 pigs, Yorkshire/Landrace cross (Midwest Research Swine, Gibbon, Minn), were randomized into two groups: the sham control group (control, 35.9 ± 1.9 kg, n = 8) and the hemorrhage with LR resuscitation group (hemorrhage, 34.0 ± 2.0 kg, n = 8). After an overnight fast, the animals were sedated with glycopyrrolate (0.1 mg/kg) and Telazol (6 mg/kg) and intubated by 1.0% to 1.5% isoflurane by mask for the surgical procedures. Polyvinyl chloride catheters were inserted into the thoracic aorta via the carotid artery for measurement of mean arterial pressure

Address reprint requests to Wenjun Z. Martini, PhD, The US Army Institute of Surgical Research, 3698 Chambers Pass, Ft Sam Houston, TX 78234. E-mail: wenjun.martini@amedd.army.mil.

This study was supported by the US Army Medical Research and Materiel Command. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of the Army or the US Department of Defense.

DOI: 10.1097/SHK.0b013e3182522e2c

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 JUN 2012	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Daily Profiles of Fibrinogen Metabolism for 5 Days Following Hemorrhage and Lactated Ringerâs Resuscitation in Pigs	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Martini W. Z., Chung K. K., Dubick M. A., Blackbourne L. H.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a REPORT	b ABSTRACT	c THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**6**	

(MAP), heart rate, and temperature. The right femoral artery was cannulated for arterial blood sampling, and the left femoral artery for the hemorrhage procedure. The left femoral vein was cannulated for LR resuscitation. The right femoral vein was cannulated for intravenous anesthesia of ketamine during the study. No splenectomy was performed in this study.

Upon completion of catheter cannulation, anesthesia was switched to a combination of isoflurane (0.5%) and continuous intravenous drip of ketamine (0.15 mL/kg per hour of 100 mg/mL) in all pigs for the remainder of the study period. After a 10 min stabilization period, MAP, heart rate, and temperature were recorded, and blood samples were taken for baseline measurements of blood gas, blood chemistry, and coagulation. To assess plasma volume, a bolus injection of sterile indocyanine green dye solution (10 mL of 2.5 mg/mL) was given, and blood samples (2 mL each) were collected at 5, 10, and 15 min after indocyanine green injection. Hemorrhagic shock was then induced in the hemorrhage group by bleeding approximately 35% of the estimated total blood volume (24.5 ± 0.1 mL/kg) over about a period of 30 min from the left femo ral artery to a preweighed canister on a balance. The rate of bleeding was con trolled by adjusting the clamp on the left femoral artery catheter to maintain MAP greater than 40 mmHg. Upon the completion of hemorrhage, the animals went through a 15 min shock period. Afterward, the pigs were resuscitated with LR solution at three times the bled volume over approximately 30 min. Pigs in the control group were not bled or resuscitated. No shed blood was returned in hemorrhaged pigs.

Upon the completion of hemorrhage and resuscitation and 15 min stabili zation, a stable isotope infusion was performed to quantify changes of fibrinogen synthesis and breakdown rates after hemorrhage and resuscitation. Sterile stable isotope solutions of 1 ^{13}C phenylalanine (1 ^{13}C phe, 100 μmol/mL) and d_5 phe (100 μmol/mL) were made in 0.45% saline and infused via the left femoral vein. A priming dose containing 1 ^{13}C phe (18 μmol/kg) and d_5 phe (18 μmol/kg) was given to the pigs, followed immediately by a constant infusion of tracer 1 ^{13}C phe (0.3 μmol/kg per minute) and d_5 phe (0.3 μmol/kg per minute). The infusion of 1 ^{13}C phe was maintained for 6 h for fibrinogen synthesis calcu lation, and the d_5 ph infusion was maintained for 3 h for fibrinogen break down calculation, in accordance with our established technique (10, 13). Blood samples (10 mL each) were collected hourly during the isotope infusion to measure fibrinogen metabolism. Additional blood samples were taken for measurements of blood gas, blood chemistry, and coagulation and plasma volume at 3 h during the infusion. Mean arterial pressure, heart rate, and temperature were recorded continuously during the infusion. Cardiac output was determined by thermodilution in triplicate at baseline, after hemorrhage and resuscitation, and at 3 h during the isotope infusion. Day 1 study was completed at the end of the isotope infusion, and all catheters inserted during the surgery procedures were taped securely on the pigs' backs. The pigs were allowed to awaken and were then transferred to an environmentally controlled room within the vivarium, where they stayed in appropriately sized runs or pens. During the night, they were fed with laboratory grade commercial swine feed by trained animal care staff. Water was provided *ad libitum* to all pigs via an automated water delivery system.

On day 2, the pigs were tranquilized with diazepam (0.5 mg/kg i.m.) before being transferred to the study room. All catheters were untied and connected to instruments as in day 1 and flushed for blood withdrawn. After 15 min stabi lization, the same 6 h isotope infusion was performed to quantify fibrinogen synthesis and breakdown on day 2. To eliminate potential leftover effect from the isotope infusion on day 1, different stable isotopes, 1 ^{13} C α ketoisocaporate (KIC, infused 6 h for calculation of fibrinogen synthesis) and d_3 KIC (infused 3 h for calculation of fibrinogen breakdown), were infused on day 2. The 6 h infusion period on day 2 coincided with that on day 1. Blood sampling and measurements were kept the same as on day 1 during the infusion. After the 6 h isotope infusion, the pigs were allowed to awaken and transferred to the viva rium in a cage for the night as on day 1.

On days 3, 4, and 5, the same 6 h isotope infusion procedures were per formed but with alternated isotope tracers to minimize potential leftover effect of tracer labeling from the isotope infusion. The isotope 1 ^{13}C phe and d_5 phe were infused on days 3 and 5, and the isotope 1 ^{13} C α KIC and d_3 KIC were infused on day 4. Blood samplings and measurements of hemodynamics, plasma volume, and coagulation were kept the same as on days 1 and 2. Upon the completion of the 6 h isotope infusion on day 5, the animals were killed with sodium pentobarbital (FatalPlus, Fort Dodge, Iowa) given intravenously by a veterinary staff member.

Calculations for fibrinogen synthesis and breakdown

Fibrinogen synthesis rate and breakdown rate were quantified based on the changing patterns of isotope tracer labeling in fibrinogen molecules during the isotope infusion. Fibrinogen fractional synthesis rates (FSRs), fractional breakdown rates (FBRs), fibrinogen absolute synthesis rate, and fibrinogen ab solute breakdown rate were calculated as previously described (10, 13).

Analytical methods

Blood gas measurements were determined by the Omni 9 Blood Gas Analyzer (AVL, Montpellier, France). Blood chemistries were measured by the Dimension Clinical Chemistry System (Dade Behring, Newark, Del). Plasma fibrinogen concentrations and D dimer levels were measured using the BCS Coagulation System (Dade Behring, Deerfield, Ill). Coagulation function was assessed in fresh whole blood samples at pig's body temperature with tissue factor as activator, using thromboelastography (TEG) (TEG 5000 Hemo stasis Analyzer; Haemoscope Corp, Niles, Ill) as described previously (14). In the TEG measurements, reaction time (*R* time) is the latency time for initial clot formation. *K* time is the duration from initial detectable clot formation to maximum clot formation. Angle (α) measures the rapidity of fibrin buildup and cross linking. Maximum amplitude (MA) represents maximum strength or stiffness of the clot, and LY_{60} indicates the percent of clot lysis at 60 min after MA is achieved.

Plasma free amino acid enrichments from the isotope infusion were deter mined following procedures described previously (10 12). Plasma fibrinogen was isolated following the procedure described by Stein et al. (15). The enrichments of phenylalanine from isolated fibrinogen were determined by gas chromatography mass spectrometry (GC MS, model 5973; Hewlett Packard, Palo Alto, Calif), as described previously (10, 13).

Statistical analysis

Data were expressed as means ± SEM and analyzed using SAS statistical software. In each group, one way analysis of variance with repeated measures using a Dunnett adjustment was conducted to compare changes to day 1 base line values. A two way analysis of variance with repeated measures using a Tukey adjustment was performed to compare the changes over time between the control and the hemorrhage groups. The statistically significant level was set at $P < 0.05$.

RESULTS

Hemodynamics

All of the animals from both groups survived to the end of the 5-day study. All baseline measurements on day 1 were similar between the control group and the hemorrhage group. No significant changes were observed in hemodynamics in the control group during the 5-day study period. In the hemorrhage group, MAP decreased on day 1 from a baseline of 95 ± 4 to 53 ± 4 mmHg ($P < 0.05$) after hemorrhage and returned to baseline following LR resuscitation. Heart rate increased from a baseline of 91 ± 8 to 129 ± 13 beats/min after hemorrhage on day 1($P < 0.05$) and returned to baseline following LR resuscitation. Cardiac output decreased from a baseline of 5.8 ± 0.3 to 2.9 ± 0.4 L/min ($P < 0.05$) after hemorrhage and returned to baseline after LR resuscitation. No significant changes in MAP, heart rate, or cardiac output occurred during the remaining study on day 1, or during day 2, 3, 4, or 5 in the hemorrhage group. No significant changes in body temperature were observed in either animal group during the study.

There were no significant changes in hematocrit or lactate levels in the control group. In the hemorrhage group, hematocrit was decreased by hemorrhage and resuscitation from baseline value of 28% ± 1% to 20% ± 1% ($P < 0.05$) on day 1 and remained at the decreased value on days 2, 3, 4, and 5. Blood lactate level increased by hemorrhage from a baseline of 1.8 ± 0.1 to 2.4 ± 0.2 mM ($P < 0.05$) after hemorrhage and returned to 1.7 ± 0.2 mM after LR resuscitation. No further changes in blood lactate levels occurred on day 2, 3, 4, or 5. There were no significant changes in base deficit or pH observed in either animal group during the 5-day study period.

Plasma volume and plasma proteins

There were no significant changes in plasma volume from a baseline value of 52 ± 1 mL/kg in the control group during the

5-day study. Plasma volume was increased after hemorrhage and LR resuscitation from a baseline of 51 ± 3 to 67 ± 4 mL/kg on day 1, remained elevated on day 2 (65 ± 5 mL/kg, both $P < 0.05$), but returned to baseline by day 3 and thereafter. There were no significant changes in plasma total protein in the control group during the 5-day study period. Plasma total protein concentration was decreased after hemorrhage and LR resuscitation from a baseline of 5.5 ± 0.3 to 4.3 ± 0.3 g/dL ($P < 0.05$) on day 1 but returned to baseline on day 2 and thereafter.

Changes in plasma fibrinogen concentrations during the 5-day study period are shown in Figure 1 (A). In the control group, plasma fibrinogen concentration did not change on day 1, rose from the day 1 baseline value of 180 ± 7 mg/dL to its peak of 340 ± 30 mg/dL on day 2 ($P < 0.05$), fell to 289 ± 19 mg/dL on day 3 ($P < 0.05$), and returned to the day 1 baseline levels on days 4 and 5. In contrast, fibrinogen concentrations in the hemorrhage group decreased after hemorrhage and LR resuscitation from a baseline of 170 ± 10 to 127 ± 17 mg/dL on day 1 ($P < 0.05$), increased to its peak (363 ± 40 mg/dL, $P < 0.05$) on day 2, and remained at this elevated level on days 3, 4, and 5. Fibrinogen content (in milligrams per kilogram), calculated by multiplying fibrinogen concentration with plasma volume, demonstrated a similar pattern as that of fibrinogen concentration (Fig. 1, B).

There were no significant changes in platelet count from the day 1 baseline value of 340 ± 35 × 10^3/μL in the control group throughout the study period. In the hemorrhage group, platelet counts decreased from a baseline of 354 ± 44 to 223 ± 16 × 10^3/μL after hemorrhage and LR resuscitation on day 1 ($P < 0.05$), remained at the lower levels on days 2 (189 ± 21 × 10^3/μL) and 3 (184 ± 16 × 10^3/μL, all $P < 0.05$), but returned to baseline values on days 4 and 5.

Fibrinogen synthesis rates

Changes of fibrinogen synthesis rates over 5-day study period are summarized in Figure 2.

Day 1: The isotope labeling of plasma phenylalanine reached plateau values in both animal groups after 1-h infusion of 1-^{13}C-phe (18.11% ± 1.45% in the control group and 14.07% ± 0.80% in the hemorrhage group). Plasma fibrinogen-bound phenylalanine labeling increased linearly during the infusion of 1-^{13}C-phe. Fibrinogen FSRs, calculated from the increasing slope of fibrinogen-bound phenylalanine labeling, was 1.57% ± 0.17%/h in the control group and 3.33% ± 0.31%/h in the hemorrhage group ($P < 0.05$ vs. control). The absolute synthesis rate, calculated by multiplying FSRs with plasma volume and fibrinogen concentration, was 1.3 ± 0.3 mg/kg per hour in the control group and 3.5 ± 0.4 mg/kg per hour in the hemorrhage group ($P < 0.05$ vs. control) (Fig. 2).

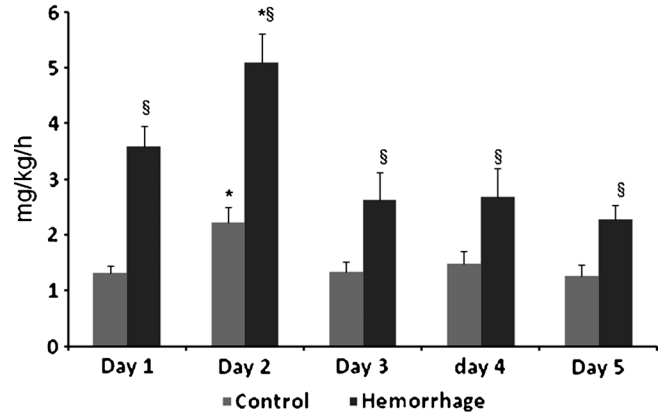

FIG. 2. **Daily changes of plasma fibrinogen synthesis rate following hemorrhage and LR resuscitation in pigs**. Data are represented as mean ± SEM for eight animals per group. $P < 0.05$ compared with day 1 baseline values within the group. $^{\S}P < 0.05$ compared with corresponding control values.

Day 2: The isotope labeling of plasma leucine reached plateau values in both animal groups after 1-h infusion of isotope 1-^{13} C-α-KIC (9.0% ± 10.7% in the control group and 11.1% ± 1.0% in the hemorrhage group). Plasma fibrinogen-bound leucine labeling increased linearly during the infusion of 1-^{13} C-α-KIC. Fibrinogen FSRs, calculated from the increasing slope of fibrinogen-bound leucine labeling, was 2.2% ± 0.3%/h in the control group and 5.1% ± 0.5%/h in the hemorrhage group ($P < 0.05$ vs. control). The absolute synthesis rate was 2.2 ± 0.3 mg/kg per hour in the control group ($P < 0.05$ vs. control day 1) and 5.1 ± 0.5 mg/kg per hour in the hemorrhage group ($P < 0.05$ vs. control and $P < 0.05$ vs. hemorrhage day 1) (Fig. 2).

Days 3, 4, and 5: Calculation of fibrinogen synthesis rates were calculated based on the increasing slope of fibrinogen-bound phenylalanine labeling on days 3 and 5, or fibrinogen-bound leucine labeling on day 4 from the infusion of 1-^{13}C-phe

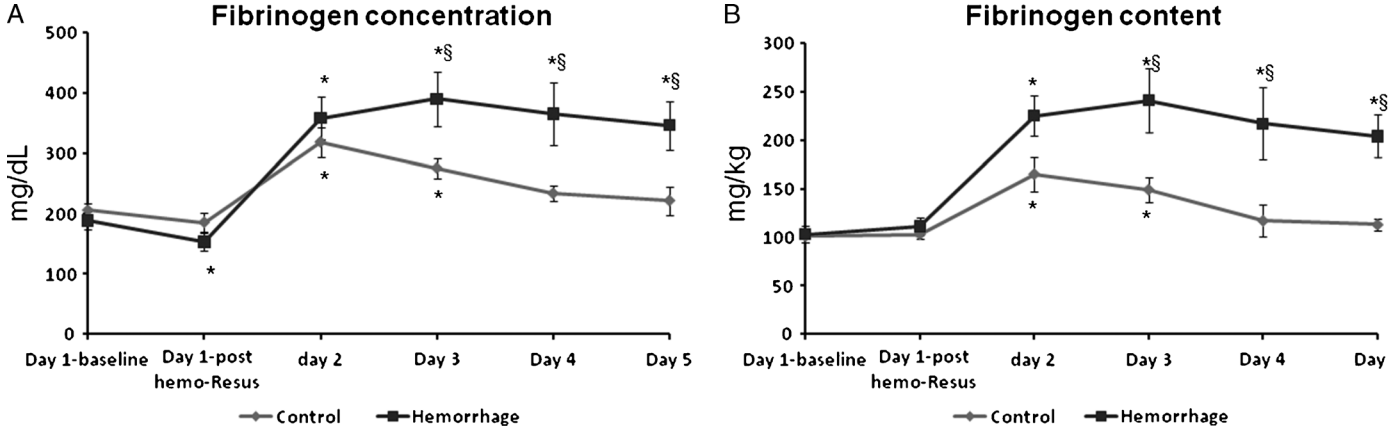

FIG. 1. **Daily changes of plasma fibrinogen concentration and content following hemorrhage and LR resuscitation in pigs**. Data are represented as mean ± SEM for eight animals per group. $^{*}P < 0.05$ compared with day 1 baseline values within the group. $^{\S}P < 0.05$ compared with corresponding control values.

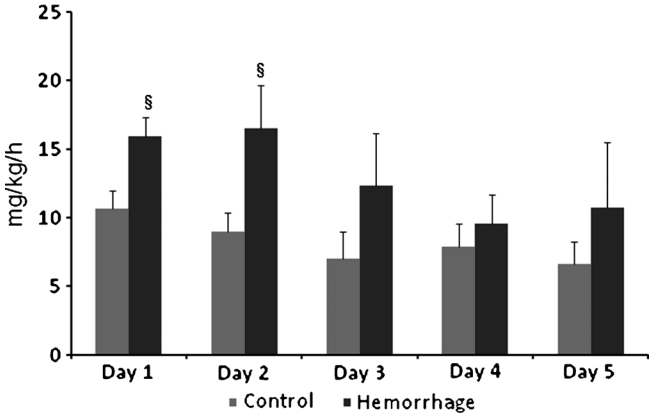

FIG. 3. **Daily changes of plasma fibrinogen breakdown rate following hemorrhage and LR resuscitation in pigs**. Data are represented as mean ± SEM for eight animals per group. $^{\S}P < 0.05$ compared with corresponding control values.

on days 3 and 5, or 1-^{13} C-α-KIC on day 4, respectively. In the control group, fibrinogen synthesis rate returned to day 1 values on days 3, 4, and 5 (Fig. 2). In the hemorrhage group, fibrinogen absolute synthesis rate decreased somewhat on days 3, 4, and 5, but remained elevated compared with corresponding control values (Fig. 2).

Fibrinogen breakdown rates

Changes of fibrinogen breakdown over 5-day study period are summarized in Figure 3.

Day 1: Calculation of fibrinogen FBR was based on the changes of fibrinogen-bound phenylalanine labeling after the cessation of the 3-h d_5-phe infusion on day 1. The calculated FBR was 12.7% ± 1.6%/h in the control group and 14.8% ± 1.1%/h in the hemorrhage group. The absolute breakdown rate, calculated by multiplying FBR with plasma volume and fibrinogen concentration, was 10.6 ± 1.3 mg/kg per hour in the control group and 16.0 ± 1.4 mg/kg per hour in the hemorrhage group ($P < 0.05$ vs. control).

Day 2: Calculation of FBR on day 2 was based on the changes of fibrinogen-bound leucine labeling after the cessation of 3-h d_3-α-KIC infusion. Fibrinogen absolute breakdown rate on day 2 did not change in the control group (8.9 ± 1.4 mg/kg per hour) but remained elevated in the hemorrhage group (16.5 ± 3.7 mg/kg per hour, $P < 0.05$ vs. control).

Days 3, 4, and 5: Calculation of FBR on days 3, 4, and 5 was calculated based on the changes of fibrinogen-bound tracer labeling after the cessation of 3-h infusion of d_5-phe on days 3 and 5 or d_3-α-KIC on day 4. In the control group, fibrinogen absolute breakdown rate remained unchanged on days 3, 4, and 5, as compared with day 1 control value (Fig. 3). In the hemorrhage group, fibrinogen breakdown rates in the hemorrhage group returned to control day 1 value on days 3, 4, and 5 (Fig. 3).

Coagulation functional changes

Compared with day 1 baseline values, there were no significant changes observed in any TEG variables in the control group during the 5-day study period. In the hemorrhage group, the initial clotting time (*R* time) did not change from baseline values by hemorrhage and resuscitation on day 1, but was prolonged on day 2, remained prolonged on days 3 and 4, and returned to baseline values on day 5 (Table 1). Time to maximum clot (*K* time) did not change from baseline values after hemorrhage and resuscitation on day 1 but was prolonged on days 2 and 3 and returned to baseline values on days 4 and 5 (Table 1). Clotting rapidity (α) did not change from baseline values after hemorrhage and resuscitation on day 1, but decreased days 2 and 3, and returned to day 1 baseline values on days 4 and 5 (Table 1). Clot strength (MA) decreased from

TABLE 1. **Changes in TEG measurements after hemorrhage and LR resuscitation (H/LR)**

	Day 1					
Measurement	Baseline	After H/LR	Day 2	Day 3	Day 4	Day 5
R time, min						
Control	3.5 ± 0.2	3.7 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.7 ± 0.2	3.7 ± 0.2
Hemorrhage	3.2 ± 0.1	2.9 ± 0.1	4.5 ± 0.3*†	4.7 ± 0.2*†	4.5 ± 0.2*†	3.5 ± 0.2
K time, min						
Control	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.0
Hemorrhage	1.1 ± 0.1	1.0 ± 0.1	1.8 ± 0.1*†	1.6 ± 0.1*†	1.3 ± 0.2	1.1 ± 0.1
Angle, α-degree						
Control	75 ± 1	74 ± 1	75 ± 1	74 ± 1	75 ± 1	76 ± 1
Hemorrhage	76 ± 1	76 ± 1	68 ± 1*†	70 ± 1*†	74 ± 1	77 ± 1
MA, mm						
Control	71 ± 1	70 ± 2	73 ± 2	72 ± 1	73 ± 1	73 ± 2
Hemorrhage	74 ± 2	66 ± 1*†	71 ± 1	72 ± 2	73 ± 2	74 ± 3
LY_{60}						
Control	6.4 ± 0.5	5.7 ± 0.6	6.2 ± 0.6	6.0 ± 0.8	6.5 ± 0.6	6.7 ± 0.6
Hemorrhage	6.0 ± 0.5	5.5 ± 0.8	4.9 ± 0.8	5.2 ± 1.0	6.0 ± 0.4	5.6 ± 1.4

*$P < 0.05$ compared with day 1 baseline values within the group. N 8/group.
†$P < 0.05$ compared with corresponding control values.

the baseline value after hemorrhage and resuscitation on day 1 but returned to day 1 baseline values on day 2 and afterward (Table 1). No significant changes in LY_{60} (fibrinolysis) were observed in either group during the 5-day study period (Table 1). Similarly, no significant changes in D-dimer levels were observed in either group during the study period.

DISCUSSION

In this study, we investigated the dynamic profile of fibrinogen concentration over 5 days after hemorrhagic shock in a swine model. As our initial effort of investigating long-term effect of hemorrhage on fibrinogen metabolism, we purposely selected a moderate degree of hemorrhage without tissue injury so all animals would survive the 5-day experimental period. In this model, fibrinogen concentration initially decreased about 25% after hemorrhage and LR resuscitation on day 1, rose to twice the prehemorrhage levels on day 2, and remained at the elevated level on days 3, 4, and 5. This dynamic profile is similar to that previously reported in a canine model (16, 17) and trauma patients (9, 18) after severe hemorrhagic shock. In dogs with hemorrhagic shock followed by LR resuscitation, Lucas et al. (16) reported that fibrinogen levels decreased after hemorrhage and resuscitation and rose the next day. In patients with hemorrhagic shock and blood transfusion, Harrigan et al. (9) reported that fibrinogen levels fell after surgery, increased and plateaued on day 2 after surgery, and remained at the plateau values even on day 25. Thus, the biphasic changes of fibrinogen concentration, with initial decrease followed by sustained increase for days, appear to be a generalized acute phase response to hemorrhagic shock and are well documented. However, it is worth mentioning that changes in fibrinogen concentration may or may not reflect changes in fibrinogen content. Fluctuations of plasma volume, which are relevant after hemorrhage and resuscitation, may change fibrinogen concentrations even when there is no change in fibrinogen content. With daily simultaneous measurements of plasma volume and fibrinogen concentration, we clarified in the current study that the increase in fibrinogen concentration after hemorrhagic shock was due to an increase in fibrinogen content.

Fibrinogen content is a dynamic balance of fibrinogen production and consumption. To our knowledge, this study is the first to investigate fibrinogen synthesis and breakdown simultaneously and independently after hemorrhage and resuscitation. A 6-h isotope infusion was performed daily for 5 days to quantify daily changes of endogenous fibrinogen synthesis and breakdown to reveal the underlying mechanisms related to changes of fibrinogen availability. Compared with the controls, fibrinogen synthesis after hemorrhagic shock was higher every day over 5 days. Fibrinogen breakdown was also higher on days 1 and 2, but returned to the control values on days 3, 4, and 5. Thus, the increase in fibrinogen content after hemorrhagic shock appears primarily due to the sustained increase in fibrinogen synthesis. Because stimulated synthesis of fibrinogen has also been demonstrated in patients with acute inflammation (19, 20), HIV (21), head injury (22), nephritic range proteinuria (23), and hemodialysis (24), the increase in hepatic synthesis of fibrinogen seems to be a generalized metabolic response after systemic insults. The underlying physiological purpose of increasing fibrinogen synthesis, however, is not fully understood but is believed to relate to its effects on coagulation as well as on the immune system (25, 26). Nevertheless, the increase in fibrinogen synthesis under stressed situation appears to be essential, because low or minimally increased fibrinogen levels were observed in nonsurvivors, whereas sustained increased of fibrinogen levels were observed in survivors of septic shock patients (27). It is possible that the stimulation of fibrinogen synthesis may reflect a necessary metabolic and physiologic compensatory effect that may relate to decreasing infection and improve survival of these patients.

Together with the increase in fibrinogen synthesis, we observed an increase in fibrinogen breakdown on day 1 after hemorrhage and resuscitation in the current study. The increase in fibrinogen breakdown was also observed in our previous acute study after hemorrhage and resuscitation (13). Similarly, an increase in the disappearance rate of radioactively labeled fibrinogen was also shown previously in a canine model at 10 h after 45% hemorrhage (28). The change of fibrinogen synthesis in that study was not clear because synthesis was not quantified (28). In the present study, both increases of fibrinogen synthesis and breakdown reflect an acute increase in fibrinogen turnover after hemorrhage and resuscitation. Along the same line, patients with infection, inflammation, burns, and trauma are characterized by an increase in whole-body protein turnover rate, with a net loss of body protein (20, 29–31). Specifically, there is an increase in amino acid release from muscle and an increase in amino acid uptake in the splenic bed (30). This shift of amino acid source from muscle to the liver is hypothesized to be beneficial as it facilitates the liver synthesis of proteins, which are critical for survival (20). The acceleration of protein turnover may facilitate this shift. In addition, in the hemorrhage group of the present study, in contrast to the sustained increase in fibrinogen synthesis, fibrinogen breakdown returned to the control value on days 3, 4, and 5. These different changing profiles of fibrinogen synthesis and breakdown suggest that fibrinogen synthesis and breakdown may be regulated via different mechanisms.

Changes in fibrinogen availability were assessed together with changes in coagulation function in this study. Prolongations of *R* time, *K* time, and decreased clotting speed (α) during days 2 to 4 in this study suggest the development of a hypocoagulable state, which might result from compromised enzymatic patterns of coagulation as well as low platelet counts after hemorrhage and resuscitation. Clot strength (MA) in TEG represents the contributions of fibrinogen and platelets to clot formation and the strength of fibrin clots, respectively. In this study, fibrinogen concentration decreased after hemorrhage and resuscitation on day 1, increased to above prehemorrhage level on day 2, and remained elevated through day 5. Clot strength decreased after hemorrhage and resuscitation on day 1 but recovered to its prehemorrhage value on day 2 and afterward. The parallel increases of fibrinogen and clot strength may suggest that clot strength is closely related to fibrinogen availability. In addition, because platelet count remained reduced

on days 2 and 3 after hemorrhage and resuscitation, the recovery of clot strength on day 2 with an elevated fibrinogen level suggests a compensatory role of fibrinogen on clot strength. Improving clot strength is associated with reductions of transfusion requirements in trauma patients (32) and 24-h postoperative blood loss in patients undergoing aortic valve operation and ascending aorta replacement (33). Thus, the compensatory effect observed of fibrinogen on clot strength in this study, despite a presumed hypocoagulable state, may support a strategy of early supplementation in patients after significant hemorrhage. Furthermore, when fibrinogen was recovered to above baseline level on day 2 and plateaued at the level afterward, clot strength was recovered to baseline value on day 2 and maintained at the level thereafter. The lack of elevation above baseline in clot strength may reflect a possible safety mechanism regulating the coagulation process after hemorrhagic shock.

In summary, we investigated the daily changes of fibrinogen metabolism and availability for 5 days after moderate hemorrhage and LR resuscitation in a swine model. Fibrinogen availability was reduced immediately by hemorrhagic shock and LR resuscitation but increased above prehemorrhage level the next day and thereafter. Fibrinogen synthesis increased for 5 days after hemorrhage and resuscitation, whereas fibrinogen breakdown increased initially but normalized on day 3. The increase in fibrinogen content was parallel with the improvement of clot strength. The compensatory effect of fibrinogen on clot strength warrants future efforts to investigate early supplementation of fibrinogen on coagulation function and fibrinogen metabolism to determine whether under such conditions as observed here there is an actual impact on control bleeding.

ACKNOWLEDGMENTS

The authors thank Douglas Cortez, Irasema Terrazas, Shavaughn Colvin, and Nahir Miranda for their technical assistance in the animal study. The authors appreciate the support received from the Veterinary Support Division and the Laboratory Support Division at the US Army Institute of Surgical Research in animal studies and coagulation measurements.

REFERENCES

1. Bellamy RF: The causes of death in conventional land warfare: implications for combat casualty care research. *Mil Med* 149:55 62, 1984.
2. Sherman LA: DIC in massive transfusion. *Prog Clin Biol Res* 108:171 189, 1982.
3. Stahel PF, Moore EE, Schreier SL, Flierl MA, Kashuk JL: Transfusion strategies in postinjury coagulopathy. *Curr Opin Anaesthesiol* 22:289 298, 2009.
4. Cotton BA, Gunter OL, Isbell J, Au BK, Robertson AM, Morris JA Jr, St Jacques P, Young PP: Damage control hematology: the impact of a trauma exsanguination protocol on survival and blood product utilization. *J Trauma* 64:1177 1182; discussion 1173 1182, 2008.
5. Hardy JF, De Moerloose P, Samama M: Massive transfusion and coagulopathy: pathophysiology and implications for clinical management. *Can J Anaesth* 51:293 310, 2004.
6. Hiippala ST, Myllyla GJ, Vahtera EM: Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates. *Anesth Analg* 81:360 365, 1995.
7. Park MS, Martini WZ, Dubick MA, Salinas J, Butenas S, Kheirabadi BS, Pusateri AE, Vos JA, Guymon CH, Wolf SE, et al.: Thromboelastography as a better indicator of hypercoagulable state after injury than prothrombin time or activated partial thromboplastin time. *J Trauma* 67:266 275; discussion 266 275, 2009.
8. Schreiber MA: Coagulopathy in the trauma patient. *Current Opin Crit Care* 11:590 597, 2005.
9. Harrigan C, Lucas CE, Ledgerwood AM: The effect of hemorrhagic shock on the clotting cascade in injured patients. *J Trauma* 29:1416 1421; discussion 1412 1421, 1989.
10. Martini WZ, Chinkes DL, Pusateri AE, Holcomb JB, Yu YM, Zhang XJ, Wolfe RR: Acute changes in fibrinogen metabolism and coagulation after hemorrhage in pigs. *Am J Physiol Endocrinol Metab* 289:E930 E934, 2005.
11. Martini WZ, Holcomb JB: Acidosis and coagulopathy: the differential effects on fibrinogen synthesis and breakdown in pigs. *Ann Surg* 246:831 835, 2007.
12. Martini WZ: The effects of hypothermia on fibrinogen metabolism and coagulation function in swine. *Metabolism* 56:214 221, 2007.
13. Martini WZ, Chinkes DL, Sondeen J, Dubick MA: Effects of hemorrhage and lactated ringer's resuscitation on coagulation and fibrinogen metabolism in swine. *Shock* 26:396 401, 2006.
14. Martini WZ, Cortez DS, Dubick MA, Park MS, Holcomb JB: Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. *J Trauma* 65:535 543, 2008.
15. Stein TP, Leskiw MJ, Wallace HW: Measurement of half-life human plasma fibrinogen. *Am J Physiol* 234:D504 D510, 1978.
16. Lucas CE, Ledgerwood AM, Saxe JM, Dombi G, Lucas WF: Plasma supplementation is beneficial for coagulation during severe hemorrhagic shock. *Am J Surg* 171:399 404, 1996.
17. Martin DJ, Lucas CE, Ledgerwood AM, Hoschner J, McGonigal MD, Grabow D: Fresh frozen plasma supplement to massive red blood cell transfusion. *Ann Surg* 202:505 511, 1985.
18. Harrigan C, Lucas CE, Ledgerwood AM, Walz DA, Mammen EF: Serial changes in primary hemostasis after massive transfusion. *Surgery* 98:836 844, 1985.
19. Jahoor F, Wykes L, Del Rosario M, Frazer M, Reeds PJ: Chronic protein undernutrition and an acute inflammatory stimulus elicit different protein kinetic responses in plasma but not in muscle of piglets. *J Nutr* 129:693 699, 1999.
20. Fleck A: Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. *Proc Nutr Soc* 48:347 354, 1989.
21. Jahoor F, Abrfamson S, Heird WC: The protein metabolic response to HIV infection in young children. *Am J Clin Nutr* 78:182 189, 2003.
22. Mansoor O, Cayol M, Gachon P, Boirie Y, Schoeffler P, Obled C, Beaufrere B: Albumin and fibrinogen syntheses increase while muscle protein synthesis decreases in head-injured patients. *Am J Physiol* 273:E898 E902, 1997.
23. Zanetti M, Barazzoni R, Garibotto G, Davanzo G, Gabelli C, Kiwanuka E, Piccoli A, Tosolini M, Tessari P: Plasma protein synthesis in patients with low-grade nephrotic proteinuria. *Am J Physiol Endocrinol Metab* 280:E591 E597, 2001.
24. Giordano M, De Feo P, Lucidi P, de Pascale E, Giordano G, Infantone L, Zoccolo AM, Castellino P: Increased albumin and fibrinogen synthesis in hemodialysis patients with normal nutritional status. *J Am Soc Nephrol* 12:349 354, 2001.
25. Mosesson MW: Fibrinogen and fibrin structure and functions. *J Thromb Haemost* 3:1894 1904, 2005.
26. Degen JL, Bugge TH, Goguen JD: Fibrin and fibrinolysis in infection and host defense. *J Thromb Haemost* 1(Suppl 5):24 31, 2007.
27. Attar S, Kirby WH Jr, Masaitis C, Mansberger AR Jr, Cowley RA: Coagulation changes in clinical shock. I. Effect of hemorrhagic shock on clotting time in humans. *Ann Surg* 164:34 40, 1966.
28. Leandoer L: Fibrinogen after massive haemorrhage. Studies on coagulation and fibrinolysis of blood and lymph in dogs. *Acta Chir Scand* 390:1 20, 1968.
29. Cuthbertson DP, Tompsett SL: Note of the effect of injury on the level of the plasma proteins. *Br J Exp Pathol*:471 475, 1935.
30. Clowes GH Jr, Randall HT, Cha CJ: Amino acid and energy metabolism in septic and traumatized patients. *J Parenter Enteral Nutr* 4:195 205, 1980.
31. Jahoor F, Desai M, Herndon DN, Wolfe RR: Dynamics of the protein metabolic response to burn injury. *Metabolism* 37:330 337, 1988.
32. Plotkin AJ, Wade CE, Jenkins DH, Smith KA, Noe JC, Park MS, Perkins JG, Holcomb JB: A reduction in clot formation rate and strength assessed by thrombelastography is indicative of transfusion requirements in patients with penetrating injuries. *J Trauma* 64:S64 S68, 2008.
33. Rahe-Meyer N, Pichlmaier M, Haverich A, Solomon C, Winterhalter M, Piepenbrock S, Tanaka KA: Bleeding management with fibrinogen concentrate targeting a high-normal plasma fibrinogen level: a pilot study. *Br J Anaesth* 102:785 792, 2009.